

Assessing the geographic intraspecific variation of endangered plant species, *Fagonia cretica* and *Tecomella undulata*

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The purpose of this study was to evaluate the intraspecific variation of two endangered plant species, *Fagonia cretica* and *Tecomella undulata*, using DNA barcodes and its function in the conservation and preservation of these therapeutic plants. *Fagonia cretica* and *Tecomella undulata* are medicinal plants utilized successfully in Desi and Unani herbal treatments. Their therapeutic characteristics make them uncommon and crucial in treating liver and stomach illnesses. As a result, these plants are rapidly explored and cut to be used as herbs. They have been classified as 'Endangered Plants' by the IUCN, and additional research is necessary. The preservation and protection of biodiversity is a current study and conversation hot-button worldwide. Specifically, from arid locations, *Fagonia cretica* and *Tecomella undulata* were collected from various parts of Punjab and Sindh. It was successful in extracting genomic DNA and amplifying it using DNA barcodes. Following sequencing, BLASTn and Clustal W tools aligned their sequences. MEGA was used for the phylogenetic analysis and the evaluation of intraspecific variation. These sequences' nucleotide variations (% divergence) showed that intraspecific variation was prevalent due to the presence of these endangered plants in various locations with various environments. The findings of this study contributed to the protection of these threatened plants and helped stop their future extinction.

Keywords: NCBI, BOLD, IUCN, Red List, MEGA, Phylogenetic Tree.

INTRODUCTION

Pakistan is in a blessed region having favorable environment for biodiversity. This biodiversity is the beauty of nature and the environment. Human activities damage the environment and the biodiversity within it (Hollingsworth *et al.*, 2008). Plants are a major part of this ecosystem and their protection is really important to save this planet from pollution and disaster. The protection of medicinal plants needs serious concern as these plants are rare and will be endangered due to their excessive usage in pharmaceutical industries (Lahaye *et al.*, 2008). The species are considered endangered when their population size reduces to 70 % and the reason for reduction is known. Intraspecific variation is a variation between the same species. It is paramount for the conservation and maintenance of species population. It helps the plants in adaptation and response to the new environment. This is an important factor in checking on the loss of species populations. If two plant species have intraspecific variation, it will be helpful for their survival and adaptation in their

changing environment. DNA barcoding is a genomic technique to identify and enlist new or existing species using genomic DNA. Also, Bioinformatics is involved in verifying the evolutionary relationship between the species.

Fagonia cretica is a medicinal plant. It belongs to the plants family, Zygophyllaceae. It is locally known as 'Dhamasa'. It is native to dry and desert regions. This plant is available in Pakistan and India. It is also present in Southern Europe, North Africa and Asia (Spampinato *et al.*, 2018). It has sour and sharp taste. It is an herb and widely used in ayurveda methodology to treat various types of diseases i.e., mouth ulcer, diarrhea, hepatitis, cough, difficulty in urination (Hussain *et al.*, 2007). It is listed as "Critically Endangered" by IUCN (The International Union for Conservation of Nature) (IUCN, 2012). The Red List of Threatened species of IUCN is the most important and comprehensive database for the global conservation status of plants, animals and fungi.

Tecomella undulata is also included in the list of medically important plants (Arshad *et al.*, 2022). It is part of Bignoniaceae, a plant family. It is an economically and

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pharmaceutically important plant. It is locally called as 'Rohida', also known as 'The State Flower of Rajasthan' (Chal *et al.*, 2011; Chhajer *et al.*, 2018). It is found in desert regions of Oman, Pakistan, Iran and India (Dhir and Shekhawat, 2012). It is well known for its valuable medicinal properties and used to treat anemia, spleen and liver diseases (Kalia *et al.*, 2014). Its population is decreasing rapidly due to its medicinal importance. Hence, it has been recorded as "Endangered." The further exploration of these endangered plant species will lead to extinction (Naz *et al.*, 2021; Qureshi *et al.*, 2016).

DNA barcoding is widely used and an effective genomic method that enables rapid and accurate identification of plants through DNA barcodes (rbcL, matK etc) (Hebert and Barrett, 2005). It is mostly applied in taxonomy, evolutionary genetics, plant pathology and different fields of molecular biology (Feng *et al.*, 2019).

This study assesses the geographic intraspecific variation of endangered plant species, *Fagonia cretica* and *Tecomella undulata*.

MATERIALS AND METHODS

Plants Sampling: The plant leaves of endangered plant species, *Fagonia cretica* and *Tecomella undulata*, were collected from different regions of Sindh and Lower Punjab, Pakistan. These were sampled from Muzaffargarh, Bhakkar, Dera Ghazi Khan, Multan, Okara, Faisalabad, Jhang and Thar desert.

DNA Extraction and PCR Analysis: The plants' DNA was extracted using modified CTAB method. The plants' leaves were collected from different regions of Pakistan. Their DNA were isolated and run-on gel electrophoresis. A PCR analysis was done with the help of DNA barcodes, *rbcL*.

Sequencing and Data Analysis: The isolated DNA were sequenced and the sequences were submitted to NCBI (National Center for Biotechnology Information) database. Also, these sequences were analyzed through the Bioinformatic tool MEGA 11. This software is widely used to analyze the evolutionary relationship between plants species applying tree construction and different models.

RESULTS

DNA Isolation: This study aimed to assess the geographic intraspecific variation of endangered plant species, *Fagonia cretica* and *Tecomella undulata*. The plants' leaves were collected from Punjab and Sindh, Pakistan. The DNA isolation was carried out using the CTAB method (Fig.1). The purified DNA was amplified through optimized PCR using DNA barcode, *rbcL*.

Amplification: The *rbcL* marker is an almost standard barcode for DNA barcoding of plants. This region is highly conserved in the chloroplast genome of all plants. That's why

this barcode can amplify and analyze all plant species. Here, *rbcL* was used along with the PCR reagent to perform PCR analysis. The thermal profile for this PCR reaction was followed as (Fig. 2).

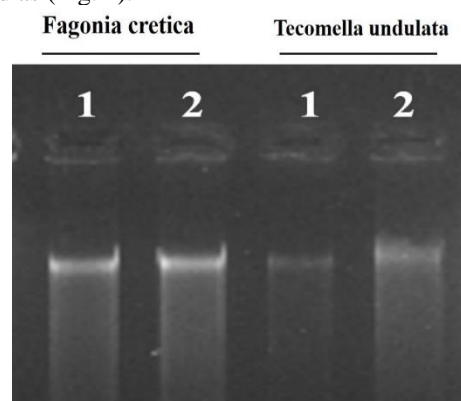


Figure 1. Isolated DNA of *Fagonia cretica* and *Tecomella undulata*.

Table 1. Thermal Profile for PCR Reaction.

Steps	Temperature	Duration
Hot start	95 °C	4 min
Denaturation	95 °C	1 min
Primer Annealing	55 °C ± 2	40 Cycles
Extension	72 °C	2 min
Final Extension	72 °C	10 min

After amplification, the amplified product was run on 1% gel electrophoresis. The amplification results were visualized under the gel documentation system (Fig. 2).

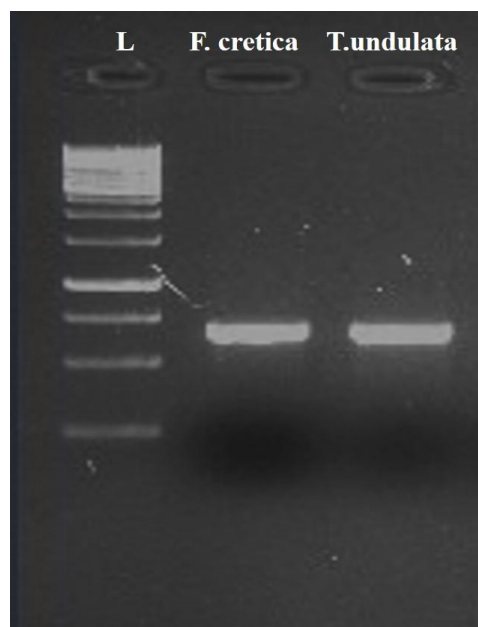


Figure 2. Amplified PCR of *Fagonia cretica* and *Tecomella undulata* using 1kb ladder (L).



Sequencing: The gel purification or elution was performed using FavorGen Gel Extraction Kit. The elution was done to cut the gel bands for further usage. After that, sequencing was done and sequences were submitted to NCBI.

Phylogenetic Analysis: After sequencing, phylogenetic analysis was performed with the help of Bioinformatic tools; BLASTn, Clustal W and MEGA 11. The examination of the evolutionary process is done using phylogenetics. This focuses on how a species changes and evolves from its predecessors. Evolution is the process of an organism's genetic divergence brought on by environmental and geographic changes.

The homology between the sequences from *Tecomella undulata* and *Fagonia cretica* was checked using BLASTn. A phylogenetic tree was produced using MEGA 11 using the Neighbor-Joining model. (Fig 3,4).

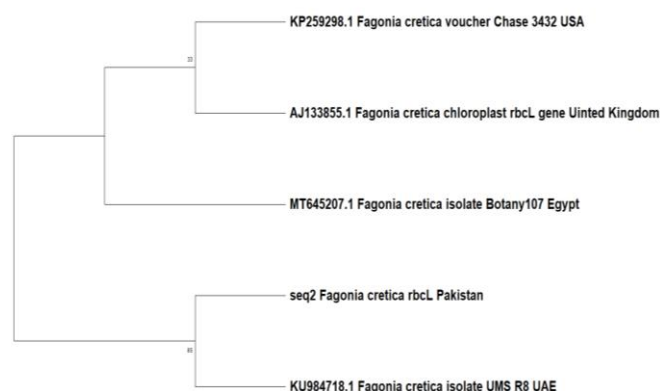


Figure 3. Intraspecific variation of *Fagonia cretica* illustrated through Neighbor-Joining (NJ) method.

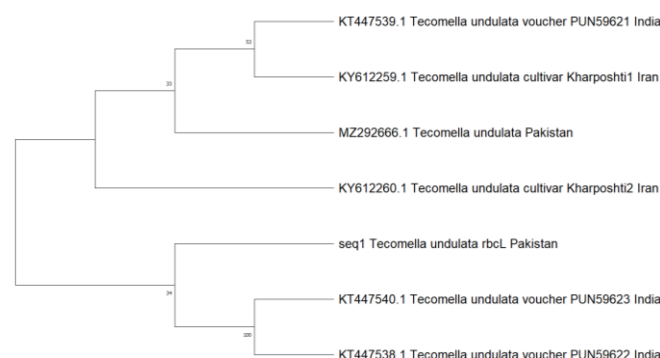


Figure 4. Intraspecific variation of *Tecomella undulata* illustrated through Neighbor-Joining (NJ) method .

The phylogenetic trees explained the intraspecific variation in the threatened plant species, *Tecomella undulata* and *Fagonia cretica*. To obtain biomolecular markers that may distinguish between species and confirm the intra-species stability or variability of these sequences, the data from this study may

be used to fill in gaps in the GenBank database. According to the statistics, *rbcL* is the gene region most effective at resolving subfamilies and supporting monophyletic or non-monophyletic genera. Strong bootstrap (BS) support allows *rbcL* to resolve the clades and display intraspecific variation within the species of *Tecomella undulata* and *Fagonia cretica*.

DISCUSSION

Medicinal plants are a crucial supply of medications to address various health issues. Pakistan is home to around 6000 distinct types of wild plants, 400-600 of which are regarded to have medicinal value. The bulk of traditional medicine used by rural Pakistanis, known as the Unani system, relies on plants or their active ingredients and is depended on by an estimated 80% of the population. The endangered plant species *Fagonia cretica* and *Tecomella undulata*, as well as their intraspecific variation, are the subject of this study. Intraspecific variation results from biotic and abiotic interactions. Plant species differ due to their occurrence in various places and environments.

Pure DNA was obtained and PCR analysis was carried out. The PCR findings indicated that using optimized DNA barcode primers, the amplification of extracted DNA was 100% effective. Because of medicinal and therapeutic chemicals, these plants are of medical relevance and are utilised as medicinal plants. These are experiencing significant population losses. They are categorized as endangered, and major conservation measures are required. This study aimed to analyze intraspecific variation in two threatened plant species, *F. cretica* and *T. undulata*. The young leaves of these plants were collected from various locations in Punjab and Sindh for this purpose. They were brought to CABB's Molecular Biology of Plant Disease Resistance Lab, where their DNA was extracted and amplified using the *rbcL* barcode. Using DNA barcoding, the *rbcL* barcode might be used to identify *Fagonia cretica* and *Tecomella undulata* successfully.

The sequences were examined using bioinformatic methods after successful sequencing. Multiple sequences were aligned using BLASTn to determine their similarity and gaps. The sequence was then compared to those found in the NCBI database. For the closest matches, the percent similarity was calculated. The query sequence's percentage of identity must be equal to or close to that of the projected species. Species identification must include in the predicted genus. MEGA 11 was used for sequence alignment and phylogenetic analysis. Using MEGA's Neighbor-Joining (NJ) cluster analysis, it was feasible to observe patterns of sequence divergence among species. As a starting point for alignment, all known nucleotide sequences of *rbcL* regions were obtained from GenBank and merged in a single file. All GenBank sequences were checked for correctness by building phylogenetic trees



for each area. MEGA 11 was used to conduct the phylogenetic analysis of *rbcl*. According to the findings, the *rbcl* gene region is the best for resolving subfamilies and supporting monophyletic or non-monophyletic genera. *rbcl* can resolve clades and reveal intraspecific variation within species of *Fagonia cretica* and *Tecomella undulata* with good bootstrap (BS) support.

Conclusion: Maintenance of the genetic diversity of forest trees is also critical worldwide for its preservation, propagation and commercial cultivation. The phylogenetic trees showed a divergence (%) from the studied endangered plants. Hence, it was proved that intraspecific variation existed between the plant species collected from different geographic ranges.

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Ethical statement: This article does not contain any studies with human participants or animal performed by any of the authors.

Availability of data and material: We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publication elsewhere.

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